A Correlation of Extracellular Superoxide Dismutase Activity with Peroxynitrite Concentration in Inhabitants around Mobile Phones Base-Stations

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Abstract

Potential health effects of radiofrequency radiation emitted from mobile phones base-stations (MPBS) have been a topic of scientific interest since the tree late decades. Earlier studies have been reported a relation of radio frequency radiation emitted from MPBS with free radical formation within the cell such as reactive oxygen species (ROS).Here, protein concentration, extracellular superoxide dismutase (EC-SOD) activity and peroxynitrite ($ONOO^-$) concentration were measured spectrophotometrically in blood plasma of inhabitants -for more than three years- around MPBS (250 m^2), then the correlation of EC-SOD activity with $ONOO^-$ concentration was investigated. The results were compared with volunteers who reside faraway from MPBS as a control group. Our results revealed presence of a significant increase in total protein concentration, EC-SOD activity and $ONOO^-$ concentration ($p< 0.05$) in comparison with that of control group. In addition, the results indicate there is no high correlation between EC-SOD activities with $ONOO^-$ concentrations. The results proved that the long exposure to emitted radiations from MPBS at domestic level has negative impact on human health due to increase the dangerous oxidant radicals which can cause many diseases and aging.

Key words: Extracellular superoxide dismutase, peroxynitrite, mobile phone base-station (MPBS), radiations.

العلاقة بين انزيم السوبراوكسيد ديسميوتيز خارج الخلية مع تركيز البيروكسي نايترايت للاشخاص القاطنين بالقرب من محطات الهواتف النقالة

علي حنين اسمير

قسم الكيمياء، كلية العلوم، جامعة ذي قار

الخلاصة

اخذت المخاطر الصحية المحتملة للاشعة المبعثة من الهواتف النقالة ومحطاتها اهتماما علميا خلال العقود الثلاثة الاخيرة. اكدت الدراسات السابقة العلاقة بين اشعة التردد الراديوي المنبعثة من محطات الهواتف النقالة وبين تكوين الجذور الحرة في الخلية الحية سيما الاصناف الاوكسجينية الفعالة. في هذا العمل، قيس طيفيا كل من تركيز البروتين، فعالية انزيم السوبراوكسيد ديسميوتيز خارج الخلية، تركيز البيروكسي نايترايت في بلازما الدم للاشخاص القاطنين جوار محطات الهواتف النقالة (حوالي ٢٥٠ متر مربع) لفترة لاتقل عن ثلاث سنوات، ثم شخصت العلاقة بين فعالية الانزيم وتركيز البيروكسي نايترايت. قورنت النتائج مع مجموعة السيطرة. نتائجنا اشارت الى وجود زيادة معنوية في تركيز البروتين، فعالية الانزيم وتركيز البيروكسي نايترايت($P< 0.05$) في بلازما دم الاشخاص القاطنين جوار محطات الهواتف النقالة مقارنة مع مجموعة السيطرة. كما اشارت النتائج الى عدم وجود علاقة كبيرة بين فعالية الانزيم وتركيز البيروكسي نايترايت في كلا المجموعتين قيد الدراسة. النتائج اثبتت ان التعرض طويل الامد للاشعة المنبعثة من محطات الهواتف النقالة المتواجدة في المناطق السكانية يمتلك تاثيرا صحيا سلبيا نتيجة تسببه في تكوين جزيئات مؤكسدة خطرة قد تسبب العديد من الامراض اضافة الى الشيخوخة.

Introduction:

There are conflicted opinions about the possible hazardous effects of exposure to radiofrequency radiations (RFR) emitted from mobile phone base-stations (MPBS) antennas. The RF covering all frequencies used for communications like radars, satellites and mobile phones. The range of this region from 300 Hz to 300 MHz [1]. In the frequency range of mobile phone radiation (900-2200 MHz), the electromagnetic radiation is non-ionizing RF energy [2].

These radiations have certain well-defined frequencies, which facilitate its discernment by a living organism, and via which the organism can, in turn, be affected. Thus some endogenous bioelectrical activities can be interfered with via oscillatory aspects of the incoming radiation changing some of biological processes in the body [3, 4].Such alteration may affect free-radical formation within the cell. Free radicals that are derived from oxygen metabolism are known as reactive oxygen species (ROS) [5].

Abbreviations:

EC-SOD: extracellular superoxide dismutase, eNOS: endothelial nitric oxide synthase, GHz: gigahertz, iNOS: inducible nitric oxide synthase, MHz: Mega Hertz, MPBS: mobile phones base-stations,NADH: nicotine amide dinucleotide, NO: nitric oxide, $O_2^{\cdot -}$: superoxide anion,$ONOO^-$:peroxynitrite, RF: radiofrequency,RFR: radiofrequency radiations, ROS: reactive oxygen species.

If ROS are not scavenged, these species may lead to widespread lipid, protein and DNA damage. Among the scavenging mechanisms in the cell are the enzymes, are which includedsuperoxide dismutase (SOD), that catalyzes the dismutation of the superoxide anion ($O_2^{\cdot -}$)into hydrogen peroxide (H_2O_2) [6]. Superoxide dismutase isoforms are classified according to the redox metal within the active site, which includes iron, manganese, copper (with a structural zinc ion) and nickel [7].

There are three isoenzymes of SOD present in mammalian cells but catalyzes the same reaction, Cu/Zn SODor SOD1 have Cu and Zn in their catalytic centre,found in the cytoplasm, nuclear compartments and in the inter membrane space of the mitochondria . While MnSOD or SOD2 has Mn in the catalytic center is localized in the mitochondria matrix of the cells. Extrecellular SOD (EC-SOD) or SOD3 have Cu and Zn in their catalytic center.

EC-SOD is the major SOD isoenzyme in extracellular fluid like plasma, lymph and synovial fluidwhere it binds the extracellular matrix through its high-affinity carboxyterminus [8,9].

In this connection, peroxynitrite ($ONOO^-$) is unstable molecule which has a half-life 1.9 seconds and at physiological pH;($ONOO^-$) is protonated to form peroxynitrous acid which can yield nitrogen dioxide and a hydroxyl-like radical. Production of ($ONOO^-$) in the body depends on $NO^\bullet$ and $O_2^{\bullet-}$ concentration which are regulated mainly by nitric oxide synthase (NOS) and SOD while the production of OH radical is one of major mechanism of ($ONOO^-$) toxicity. $ONOO^-$can directly oxidize transition metal (Fe, Mn, Cu) in the active center of the enzymes, it can nitrate the tyrosine residues in different proteins that present in the site and oxidize thiols in vivo and in vitro, and also $ONOO^-$ induces both apoptosis and necrosis of cells [10-12]. There are no enough findings in the literatures illustrate the effect of MPBS radiation on the $ONOO^-$ concentration. The aim of this work was to investigation of MPBS radiations impact on EC-SODactivity and $ONOO^-$concentration in blood samples of inhabitants around MPBS, then determination of EC-SOD correlation with $ONOO^-$.

2. Subjects and Methods:

The study was performed on group of individuals who reside nearby MPBS (within 250 m^2) in different areas of Baghdad city at least for three years, compared with control group for individuals who reside faraway from these MPBS. All individuals were healthy males; aged 25±5 years and nonsmokers.

2.1 Materials:

All chemicals werehigh qualityobtained from BDH and FLUKA companies.

2.2 Blood Sample Collection

Five milliliters of venous blood samples were collected from individuals reside nearby MPBS(exposed group) and control group into EDTA-tubes which were then centrifuged at 3000 g for 10 minutes; then plasma was aspirated carefully by Pasture pipette. Samples were stored frozen until used to estimate the parameters.

2.3. Determination of Protein Concentration:

Plasma proteins of all samples were determined by simple Lowry's method[13].

2.4 Determination of SOD activity:

Superoxide dismutase (SOD) activity was determined according toriboflavin/NBT method[14].

2.5 Determination of Peroxynitrite Concentration:

The peroxynitrite mediated nitration of phenol was measured spectrophotometrically according to the method of Vanuffelen[15].

2.6 Statistical Analysis:

Data were expressed as the mean ± S.D. and statistically analyzed for significance using the analysis of variance single factor model followed by a two-tailed Student's t-test. Means are presented in bar charts, with error bars indicating standard deviations; $p< 0.05$ was regarded as statistically significant.

3 Results and Discussion

3.1 Protein Concentration:

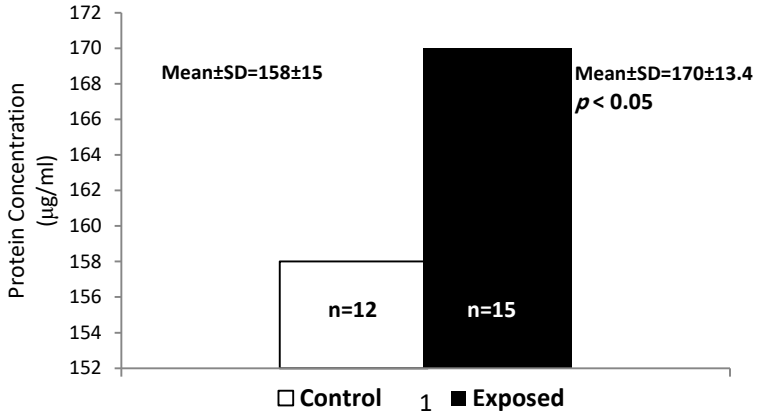

Figure 1: Mean values and standard deviations of protein concentration (µg/ml).

The results in ***Figure1*** reveal presence of a significant increase ($P< 0.05$) in plasma protein concentration of exposed group compared with that of control group. In previous study, it has be found that protein concentration of red blood cells of individuals who living near mobile phone stations was higher than in control group [16].

These results comein agreement with study of Gaafar*etal*., who reported that exposed *E.coli*cells to electromagnetic radiation had higher protein concentration [17]. Karinen*et al.* reported that protein expression in human skin might be affected by the exposure to RF-EMF from mobile phone [18]. Results of the presented study disagree with Kula*etal.* who found that significant decreases in the levels of total protein in serum of steel workers exposed to electromagnetic field [19], and also disagree with El-Abiad*et al.* who showed a significant reduction in total serum protein in old rats exposed to radiation from MPBS [20]. In addition, our results opposite with Hassan who observed significant decrease in total protein concentration in rats exposed to electromagnetic field [21], while Abed found that no significant difference in protein concentration for individuals exposed to mobile radiation [22].

Increased in all, groups, or an individual protein can be caused increased protein concentration. Among these proteins a group of several proteins called positive acutephase reactants like α1- antitrpsin, α1- acid glycoprotein, C-reactive protein and ceruloplasmin, which were reported to increase significantly during some conditions [23- 25].The observed increase may be due to increased synthesis of stress proteins or heat shock proteins (HSP (such as hsp70)) which are induced by a variety of potentially harmful extracellular stimuli. The earlier studies showed that electromagnetic radiations induce heat shock protein [26-29].Authors studied the effects of RF on calcium dynamics in stem cell-derived neuronal cells and discovered a significant increase in intracellular calcium spikes in response to non-thermal RF. These studies suggest that the plasma membrane might be the target of RF; this can be explained as being a result of membrane leakage which changes in electrolyte and nonelectrolyte permeability, then modifications in the proteins and lipid matrix of the membrane will occur [30, 31].

3.2 EC-SOD Activity:

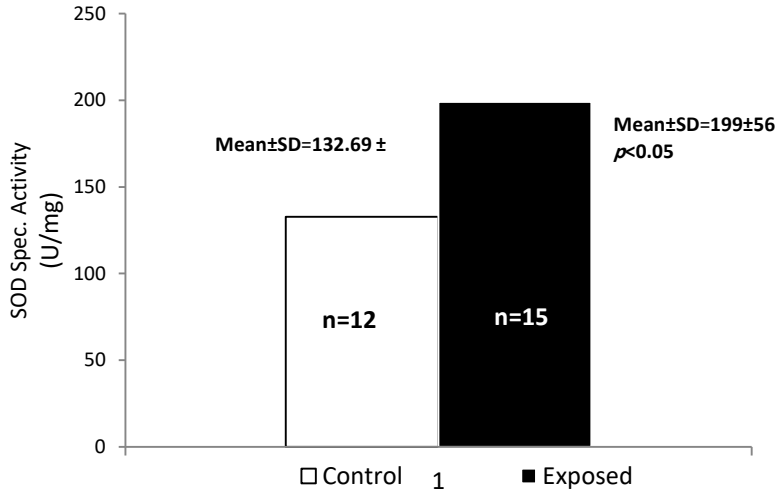

Figure 2: Mean values and standard deviations of EC-SOD specific activity (U/mg).

The results as shown in ***Figure2*** reveal high significant increase in EC-SOD specific activity ($P< 0.05$) in plasma of exposed group in comparison to that of the control group. The present results agree with that of study conducted by Kula*etal*, which pointed that activity of EC-SOD increased of rats exposed to magnetic fields [19]. Also agree with some studies which reported asignificantincrease in SOD activity in animals exposed to mobile phone radiation [32-34].

In vitro, microwaves produced by mobile phones significantly depleted SOD activity in

human bloodplatelets after exposure to radiation[35], but other author indicated that RF radiation did not alter SODinJ774.16cells [36]. On the other hand, the present results were in contrast with results of study which reported that activity of SOD inhuman erythrocytes was significantly decreased after exposure to radiofrequency fields of the mobile phone [37].Other researchers found SOD activity decreased significantly in the animal tissues or cells exposed to mobile phone radiation or radiation with same frequency [38-42]. One important point should be considered when someone look at the disagreement in the results among different reports is the differences in technical features of used devices in the experiences. Production of $O_2^{\bullet-}$by plasma membrane NADH oxidase activity stimulated byRF had been reported [43].

Superoxide dismutase (SOD) metabolizes superoxide radical ($O_2^{\bullet-}$) and dismutases it to hydrogen peroxide (H_2O_2), and protects the cell against $O_2^{\bullet-}$ mediated lipid peroxidation [44]. Since there is an enhancement of free radical activity that causes endothelial damage, the body raises the level of its antioxidants in order to combat such oxidative stress or oxidative damage [45]. Dismutation of increased superoxide radicals, in particular, can be achieved by high SOD activity [46].The frequencies of atoms groups oscillation in the active center of an enzyme are located in the range of 10-100 GHz. The approximate resonant frequencies in Hz have been determined experimentally for a few structures in living cells [47]. MPBS radiations induce free radical formation in some tissues has been reported [32, 48] but the direct biological effects of exposure to 900MHz RF radiation have not been studied extensively. We know that biochemical reactions which involve more than one unpaired electron will be affected by a magnetic field [49]. It seems to be difficult to understand the implicit mechanism for radiation related oxidative stress. The results in the present study suggest that ROS were generated under the experimental conditions employed. The observed increased activity may be caused by two some factors such as increased expression of SOD gene and/or changes in physical properties of SOD. Some of post-translational modifications may be change SOD activity [50]. One study has demonstrated that alkylation of CuZn SOD enhances its structural stability [51] this may lead to dimensioned rate of enzyme degradation; thus, resulting in a higher concentration of the enzyme being present in the cells. Another possibility is that the greater stability of the SOD enzyme might lead to an elevated activity of the enzyme [50]. Modification of the catalytic activity of an enzyme by allosteric effectors is well established in enzymology [52].

3.3 PeroxynitriteConcentration:

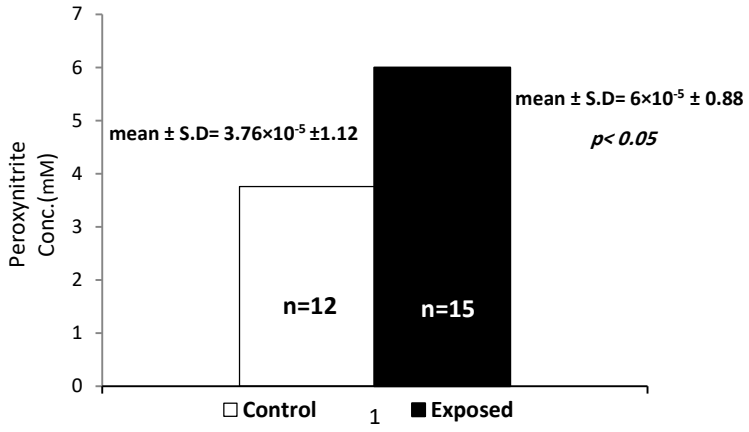

Figure 3: Mean values and standard deviations of peroxynitrite concentration (mM).

The results in the ***Figure3*** indicate that high significant increase in $ONOO^-$ concentration in plasma of exposed group compared with that of control group (P<0.05). As it said previously, the literatures survey didn't provide us with findings illustrate the effect of mobile phone radiation on the $ONOO^-$concentration.It is a clear fact that $ONOO^-$ results from $NO^\bullet$ and $O_2^{\bullet-}$ rapid reaction. [53, 54].This reaction occurs at a rate of 6.7×10^{9} M^{-1} s^{-1} which is approximately three-times faster than the reaction between $O_2^{\bullet-}$ and the SOD [55]. Likewise, the reaction between both radicals released by endothelial cells is limited by the rate of diffusion of the radicals themselves. Control of the $O_2^{\bullet-}$ and $NO^\bullet$ reaction may be the function of the EC-SOD enzymes which bind to endothelial cell surfaces [56]. Earlier studies revealed that mobile phones radiations might increase the production of $NO^\bullet$ and $O_2^{\bullet-}$. However, in case of chronic oxidative stress, once inducible nitric oxide synthase is totally activated, $ONOO^-$ will generate. Treatment of human endothelial cells with UV radiation resulted in an increase of both $NO^\bullet$and $ONOO^-$ release. The amount of $NO^\bullet$ released by UV-irradiated endothelial cells in the presence of SOD was much higher than in its absence, suggesting the neutralization of $NO^\bullet$ by $O_2^{\bullet-}$ with subsequent formation of $ONOO^-$. However, in case of chronic oxidative stress, once inducible nitric oxide synthase is totally activated, ordinary antioxidants provide little protection due to massive $ONOO^-$ generation [43, 57,58]. Antioxidants successively compete with NO for $O_2^{\bullet-}$; as a result, high $ONOO^-$levels follow [59].Therefore, when both $O_2^{\bullet-}$ and NO are generated within a few moleculardiameters of each other, they combine spontaneously toform $ONOO^-$ in a diffusion-limited reaction. Reports presumed that initially ROS production reduced the endothelial NOS (eNOS)-derived NO within endothelial cells while activating *i*NOS (iNOS) which causes almost a 1,000-fold higher NO production than *e*NOS does under physiologic circumstances [12].

3.4 The correlation between EC-SOD activities and $ONOO^-$concentrations:

Among the aims of this work, one aim was to determine the relation of EC-SOD activity with ONOO−concentrationin exposed group andcontrol group. A correlation curve was plotted between EC-SODactivities with ONOO−concentrations.

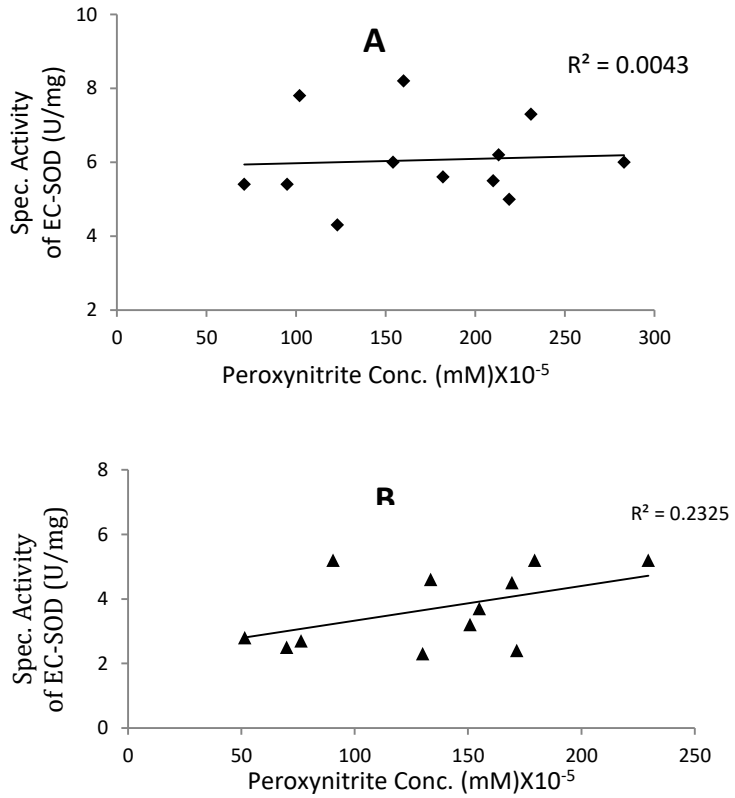

Figure 4: The correlation betweenEC-SOD activities with ONOO− concentrations.
A: Exposed group B: Control group

From the results in ***Figure 4(A,B)***, it is clear that there was no strongly correlation between EC-SOD activities with $ONOO^-$ concentrations in both studied groups. A very weak positive correlation between EC-SOD and $ONOO^-$concentrations ($R^2 = 0.0043$) in exposed group, while a weak positive correlation in control group ($R^2=0.2325$).

4. Conclusions:

The results proved that the long exposure to emitted radiations from base-stations of mobile phones at domestic level has negative impact on human health due to increase the dangerous oxidant radicalswhich can cause many diseases and aging.

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